# Research Journal of
# Phytochemistry

ISSN 1819-3471

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OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2017.85.89

# Research Article
# Antioxidant Properties and Phenolic Compounds in Methanolic Extracts of *Eichhornia crassipes*

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## Abstract

**Background and Objective:** Natural antioxidants are responsible for preventing the deleterious consequences of oxidative stress. Plants not only provide food for man but also provide number of active compounds with potent and varied therapeutical value. The present study was carried out to evaluate the antioxidant activity and quantitative estimation of the total phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total flavonoid as Quercetin Equivalent (QE) per gram dry weight of *Eichhornia crassipes* (Pontederiaceae) from methanolic extract of different plant parts (Leaves, petiole and roots). **Materials and Methods:** The methanol extract of the leaves, petiole and bark of *Eichhornia crassipes* were estimated for total antioxidant capacity using 1,1-diphenyl picrylhydrazyl (DPPH) free radical scavenging assay, total phenolic and flavonoid contents using spectrophotometric methods. Statistical analysis was performed as Mean±Standard Error Mean (SEM). The $IC_{50}$ values were also calculated by linear regression analysis. **Results:** Maximum total phenolic content was recorded in leaves ($IC_{50}$ value 0.217±0.032 mg $g^{-1}$ dry weight) and maximum total flavonoid content was found in leaves ($IC_{50}$ value 0.481±0.023 mg $g^{-1}$ dry weight) and highest radical scavenging activity was observed in petiole with $IC_{50}$ value 6.411±0.46 mg $mL^{-1}$ compared with standard values obtained from gallic acid standard $IC_{50}$ value 0.516±0.22. **Conclusion:** It is concluded that the phenolics and flavonoids represent good sources of natural antioxidants. The above results depicted that this plant exhibits significant antioxidant activity.

Key words: Quericitin, gallic acid equivalent, quercetin equivalent, spectrophotometric methods, *Eichhornia crassipes*, therapeutical, antioxidant activity

Received: February 05, 2017 Accepted: March 07, 2017 Published: March 15, 2017

Citation: Tulika Tyagi and Mala Agarwal, 2017. Antioxidant properties and phenolic compounds in methanolic extracts of *Eichhornia crassipes*. Res. J. Phytochem., 11: 85-89.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Eichhornia crassipes* (family: Pontederiaceae), a native of South America, is one of the free floating macrophytes found in the aquatic environment such a ditches, ponds and lakes. It is an herbal product rich in phytochemicals[1]. It is listed as one of the most productive plants on earth and is considered one of the top 10 world's worst aquatic plants[2]. Water hyacinth is a source of many compounds with radical-scavenging activity, such as vitamins, terpenoids, phenolic acids, lignin, stilbenes, alkaloids, sterols and other metabolites with high antioxidant activity[3]. Phenolic compounds have therapeutic potential against different diseases because of their antioxidant property. They are known to possess antispasmodic, antiviral, anti-inflammatory, antisecretory, antiulcer, antidiarrheal and antitumor activities. Flavonoids are a group of polyphenolic substances present inmost plants and are responsible for various biochemical and antimicrobial activities. They exert their antioxidant activity via radical scavenging, metal ion chelation and membrane protective efficacy[4,5].

Free radicals or Reactive Oxygen Species (ROS) or Activated Oxygen Species (AOS) are produced as by product of normal metabolism also by xenobiotic compounds, drugs or ionizing radiations. Plants also generate ROS as signalling molecules to control various processes such as programmed cell death, pathogen defence and stomatal behavior. The ROS or free radicals are highly toxic which cause damage to genetic material and lipid peroxidation and also inactivate membrane bound enzymes. It also cause chronic and degenerative disease like Alzheimer, aging, pulmonary disease, cardiovascular disease, cancer and rheumatoid arthritis[6]. Many medicinal plants have great antioxidant potential, antioxidants reduce oxidative stress in cells and therefore are useful in treatment of disease like cancer, cardiovascular and inflammatory diseases[7].

Antioxidants are the compounds which possess the ability to protect the cell organelles from damage caused by free radicals induced oxidative stress either by inhibiting the initiation or propagation of oxidative chain reactions[8,9]. Antioxidants exhibit their antioxidant activity either by inhibiting lipid peroxidation, by scavenging free radicals and active oxygen species, preventing the decomposition of hydrogen peroxides into free radicals or by chelating heavy metal ions[10,11]. The study revealed the antioxidant activity and the total phenolics and flavonoid of *Eichhornia crassipes* (Pontederiaceae) from methanolic extract of different plant parts that showed therapeutically and pharmacological importance of ethanomedicinal properties.

## MATERIALS AND METHODS

**Plant material:** The different plant parts (Leaves, petiole and root) of *Eichhornia crassipes* were collected from different areas of Kota, Rajasthan, India. It was washed with distilled water, dried at room temperature and ground to fine powder.

### Total phenolic and flavonoidal content

**Plant extraction:** Two grams each of the dry material (Leaves, petiole and root) were extracted with 50 mL of methanol at room temperature for 48 h, filtered through Whatman paper No. 1 filter paper, stored and used for quantification.

**Total phenolic content:** Total phenolic compound contents were determined by the Folin-Ciocalteu method[12,13]. The extract samples (0.5 mL, 1:10 diluted) were mixed with Folin Ciocalteu reagent (1.5 mL, 1:10 diluted with distilled water) for 5 min and aqueous $Na_2CO_3$ (4 mL, 1 M) was then added. The mixture was allowed to stand for 30 min and the total phenols were determined by colorimetric method at 765 nm. The standard curve was prepared using the standard solution of gallic acid in methanol in the range 0.2-1 mg $mL^{-1}$.

Total phenol values are expressed in terms of gallic acid equivalent (mg $g^{-1}$ of dry mass), which is a common reference compound. Total phenolic content can be calculated from the Eq. 1:

$$T = \frac{C.V}{M} \quad (1)$$

Where:

T = Total phenolic concentration
C = Concentration of gallic acid from caliberation curve (mg $mL^{-1}$)
V = Volume of extract (mL)
M = Weight of methanol plant extract

**Total flavonoid content:** Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) with slight modifications using quercetin as standard[14]. Approximately 0.5 mL of test material was added to 50 mL volumetric flask containing 3 mL of methanol. To above mixture, 2 mL of 10% $AlCl_3$ was added. After 5 min, the total volume was made up to 5 mL with methanol. Then the solutions were mixed well and absorbance was measured against blank at 420 nm. The standard curve was prepared using the standard solution of quercetin in methanol in the range 0.2-1 mg $mL^{-1}$ ($R^2 = 0.991$). Total flavonoid content of

the extracts was expressed in milligram of quercetin equivalents per gram dry weight. Total flavonoid content can be calculated from the Eq. 2:

$$T = \frac{C.V}{M} \quad (2)$$

Where:

T = Total flavonoid concentration
C = Concentration of quercetin from caliberation curve (mg $mL^{-1}$)
V = Volume of extract (mL)
M = Weight of methanol plant extract

### Determination of antioxidant activity

**DPPH assay:** Antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The diluted working solutions of the test extracts were prepared in methanol. Gallic acid was used as standard in solutions ranging from 0.5-4.0 µg $mL^{-1}$. Approximately 0.135 mM DPPH solution in methanol was prepared. Then 2 mL of this solution was mixed with 2 mL of sample solutions ranging for *E. crassipes* leaves extract 0.265-1.06 mg $mL^{-1}$, *E. crassipes* petiole extract 2.2-8.8 mg $mL^{-1}$, *E. crassipes* root extract 1.464-4.392 and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a UV-Vis spectrophotometer against methanol as blank. The control was used is 2 mL of methanol with 2 mL of DPPH solution. The optical density was recorded and percentage of inhibition was calculated using the Eq. 3:

$$\text{Inhibition of DPPH activity (\%)} = \frac{A-B}{A} \times 100 \quad (3)$$

where, A is optical density of the control and B is optical density of the sample.

**Statistical analysis:** Experimental results are expressed as Mean±Standard Error Mean (SEM). All measurements were replicated three times. $IC_{50}$ values were also calculated by linear regression analysis using GraphPad prism statistical software[15].

## RESULTS AND DISCUSSION

**Phenolic and flavanoid contents:** The phenolic and flavonoid content of the ethanolic leaves, petiole and root extract were calculated according to method discussed above (Table 1).

Table 1: Quantitative analysis of total phenolic and flavonoids content in different plant parts of *Eichhornia crassipes*

| Plant parts | Total phenolic content (mg GAE $g^{-1}$ dry weight) | Total flavonoidal content (mg QE $g^{-1}$ dry weight) |
|---|---|---|
| Leaves | 0.217±0.032 | 0.481±0.023 |
| Petiole | 0.180±0.031 | 0.27±0.22 |
| Root | 0.187±0.014 | 0.389±0.025 |

Each value is expressed as Mean±SEM (n = 3):

$$SEM = \frac{SD}{\sqrt{n}}$$

where, SD is standard deviation, SEM is standard error mean, n is No. of set

Table 2: DPPH radical scavenging assay of different plant parts of *Eichhornia crassipes*

| Plant parts | $IC_{50}$ values (mg $mL^{-1}$) |
|---|---|
| Leaves | 0.742±0.02 |
| Petiole | 6.411±0.46 |
| Root | 4.324±0.54 |

Standard (Gallic acid) 0.516±0.22, each value is expressed as Mean±SEM (n = 3):

$$SEM = \frac{SD}{\sqrt{n}}$$

where, SD is standard deviation, SEM is standard error mean and n is No. of set

Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoid, including flavones, flavonols and condensed tannins are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidant *in vivo*.

**Antioxidant activity:** The effect of antioxidant on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Extracts of plants are allowed to react with the stable radical, DPPH in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% ($IC_{50}$) is a parameter widely used to measure the antioxidant activity. A lower $IC_{50}$ value corresponds with a higher antioxidant power. The leaves, petiole and root extract show significant DPPH scavenging activity compared with the values obtained for gallic acid standard. All results are shown in Table 2 and Fig. 1.

The present study showed useful vital antioxidant for food and pharmaceutical industry. The antioxidant activity of

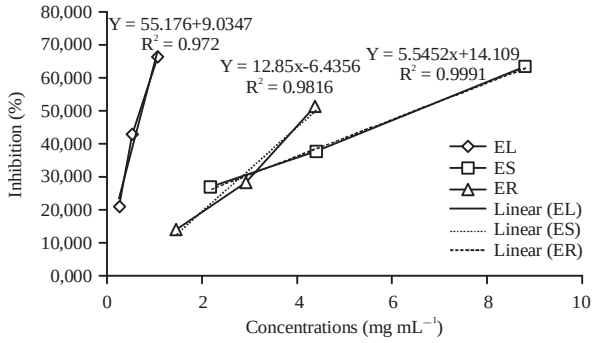


Fig. 1: DPPH radical scavenging assay of different plant parts of *Eichhornia crassipes*

*Eichhornia crassipes* by DPPH assay found their scavenging activity. Phenolic and flavonoids content have shown a good correlation with antioxidant activity, this may be due to structural differences. The $IC_{50}$ value in milligram per milliliter of each extract was found correlated with higher concentration of polyphenols and flavonoids. Extract of different parts of plant having lesser $IC_{50}$ values are more potent antioxidant and simultaneously showed the increased concentration of polyphenols and flavonoids.

Reducing power assay of ethanol fractionate of dry *Eichhornia crassipes* (Mart.) Solms at different concentrations and time delay showed that the absorbance increased with time and concentration[16]. The reducing power of petroleum ether, ethyl acetate, acetone and hydrolyzed extract of water hyacinth determined by ferricyanide method showed that the extracts of water hyacinth possess good antioxidant activity[17]. The antioxidants of plant origin with free-radical scavenging properties have great importance as therapeutic agents in several diseases caused due to oxidative stress[18]. Many synthetic antioxidant compounds have shown toxic and or mutagenic effects, which have stimulated the interest of many investigators to search natural anti-oxidants[19]. It is demonstrated that the higher antioxidant activity of water hyacinth may be due to synergistic effect of its components, chlorophylls, carotenoids and phenolic compounds, results shows to provide rich source of natural bioactive compound with antimicrobial, antitumoral, antiviral and antioxidant activities[20].

The ethanolic extract of *E. crasspies* contained high amount of phenolic acids, whereas, water extracts contained less amount of a varied number of phenolic acids, therefore water hyacinth leaves extracted a high $Fe^{2+}$ chelating activity and inhibited lipid peroxidation process both in liposome and fish oil[21]. According to the previous study[22] phenol contents contribute significantly to the total antioxidant activity of plants. It has been reported that *E. crassipes* as a safe cancer medicine and revealed its tumor inhibition potential[23]. Leaves of water hyacinth contain antioxidizing agents and glutathione and this antioxidant was determined by an enzymatic assay using glutathione reductase. Methanol leaf extract[24] and ethanol extract of water hyacinth showed good DPPH radical scavenging ability and ethanol extract of water hyacinth shows good reducing capacity[25]. Phenols, flavonoids[26,27], tannins[27], alkaloids and glycosides[26] are good antioxidant substances and prevent or control oxidative stress related disorders. Water hyacinth contains flavonoids, alkaloids, tannins, glycosides and phenols[28]. Results suggested that *E. crassipes* is potencial source of antioxidant activity and could be used as an antioxidant and preservative in food and non-food systems.

## CONCLUSION AND FUTURE RECOMMENDATIONS

It is concluded that the investigated plant *E. crassipes* showed very good antioxidant activity for DPPH assay. It formed a good base for further research on the antioxidant ability of the plant use as a commercial medicine for the free radicals related diseases. This study showed that the phenolic and flavonoidal content also have remarkable effect on antioxidant activity.

## SIGNIFICANCE STATEMENTS

This study show significant antioxidant values and total polyphenol and flavonoids concentration, which can be correlated on the basis of concentration of $IC_{50}$ values. This study discovered that leaves of *E. crassipes* have less $IC_{50}$ value which showed increased concentration of polyphenol and flavonoids which act as good antioxidant while petiole and root of *E. crassipes* showed high $IC_{50}$ values which signify less potent antioxidant and has good concentration of polyphenol and flavonoids.

## ACKNOWLEDGMENT

The authors are grateful to Mr. Naveen Garg and Shabhir Khan, S.R. Labs and Research Center, Jaipur, Rajasthan for providing laboratory facilities and assistance for antioxidant activity.

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